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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference LPB/P100255WO	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/04798	International filing date *(day/month/year)* 05.11.2003	Priority date *(day/month/year)* 06.11.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant UNIVERSITY OF LEEDS et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
26.05.2004	31.03.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Costa Roldán, N Telephone No. +49 89 2399-7180

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/04798

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-32 as originally filed

Sequence listings part of the description, Pages

1-11 as originally filed

Claims, Numbers

1-31 received on 01.03.2005 with letter of 01.03.2005

Drawings, Sheets

1-10 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 19-27 (full), 7,29(in part), 15-18 (IA)

   because:

   ☒ the said international application, or the said claims Nos. 15-18 (IA) relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 19-27(in full), 7, 29(in part)

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☒ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-18,28-31 (inventions 1, 61, 38 and 74) .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-18,28-31
	No:	Claims	
Inventive step (IS)	Yes:	Claims	31
	No:	Claims	1-18,28-30
Industrial applicability (IA)	Yes:	Claims	1-14,28-31
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 15 to 18 relate to methods of treatment and or diagnosis and are therefore considered by this Authority to be covered by the provisions of Rule 67.1 (iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of these claims (see also Art. 34(4)(a)(I) PCT).

Claims for which no International Search Report has been established have not been examined (see Rule 66.1(e)PCT). Therefore, an opinion is provided with respect to the provisions of Article 33(1) PCT (i.e. novelty, inventive step and industrial applicability) for **only for claims 1-18 and 28-31** (insofar they relate to inventions 1, 38, 61 and 74).

Re Item IV

Lack of unity of invention

The common concept which could link inventions 1 to 106, as required by Rule 13.1 PCT, could be seen as the provision of nucleic acid ligands (RNA aptamers) to fibrillar protein targets and target sequence therefor. This link cannot be considered as a single inventive concept in the sense of Rule 13.2 PCT because this common concept is known from documents D1 and D4.

Document D1 (Ylera et Al., see p. Abstract, p. 1587, left-hand-column) relates to RNA aptamers specific for fibrillar amyloid protein Beta-A4(1-40).

Document D4 (DE-A1-19916417, see p. 3, l. 50-51, and cls.1-2) relates to RNA aptamers specific for amyloid proteins, namely Beta-A4(1-40) and Beta-A4(1-42).

Thus, the technical problem to be solved by the present application may be regarded as providing alternative RNA aptamers and targets to those of D1 and D4.

In view of the fact that RNA aptamers designed for fibrillar proteins are known (see D1 and D4), that specific nucleic acid ligands have been designed for any target molecule (see D1, abstract) and that to designed RNA aptamers can be routinely applied for any other form

of fibrillar protein of interest (e.g. a fibrillar form present in an amyloid disease); the additional feature of RNA aptamers binding to an alternative form of the fibrillar protein (e.g. monomeric, pre-fibrillar, proto-fibrillar, mature or immature form) is not considered to have an inventive character. No other feature links the different RNA aptamers, therefore each of the RNA aptamers to which the application relates (namely, RNA aptamers that bind the fibrillar protein Beta-A4(1-40) and RNA aptamers that bind the fibrillar protein Beta-2-microglobulin) represents a separate solution to the above identified problem.

Hence, the provision of RNA aptamers specific to fibrillar protein target cannot, as such, represent the special technical feature as defined in R. 13.2 PCT.

Sequences with SEQ ID NOS: 111 to 113 (see description p. 9, l. 30 to p. 10, l. 9) are peptide sequences derived from human Beta-2 microglobulin used as target sequences for RNA aptamers (namely, aptamers binding motifs) of the present invention. However, the application does not specify which of the aptamers with SEQ IDS NO: 58 to 105 binds to which of the peptide sequences with SEQ ID NOS: 111 to 113. Therefore, it is not possible to group together said aptamer sequences with any of said target sequences.

Thus, the afore listed inventions are no longer linked by a special technical feature (see also Rule 13.1 PCT). The requirements with respect to unity of invention, are, thus, not fulfilled and the claims relate to the 106 different inventions listed above.

Under protest, additional fees were paid for the search of a single further invention. After review of the protest, the International Searching Authority (ISA) decided to search two further inventions, in total 4 inventions were searched (namely, inventions 1, 38, 61 and 74). The IPEA agrees with the ISA in respect to the lack of unity of the inventions. However, **an opinion about novelty, inventive step and industrial applicability is made for all the inventions searched (inventions 1, 38, 61 and 74).**

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: Ylera et Al., Biochemical and Biophysical Research Communications, 2002, Vol 290, p. 1583-1588.

D2: WO-A-0188123.
D3: DE-A1-19916417.

V.1. INVENTION 1:

V.1.1. NOVELTY (Article 33(1) and (2) PCT)

The prior art does not describe a non-naturally occurring RNA ligand to D-amino acid A-Beta1-40 monomeric target protein consisting of the nucleic acid sequence with SEQ ID NO: 1, a vector comprising said RNA ligand, a host cell comprising said vector, a pharmaceutical composition comprising said RNA ligand, the use of said RNA ligand for manufacture of a medicament, and a method for the isolation of said RNA ligand. Therefore, **claims 1-18** (insofar they relate to invention 1) are **novel** (Art.33(1)(2) PCT).

V.1.2. INVENTIVE STEP (Article 33(3)PCT)

The subject-matter of claim 1 differs from the disclosure of D1 in that the RNA ligand (aptamer) to A-Beta1-40 target protein, consisting of SEQ ID NO: 1, is not mentioned in D1.

Hence, the problem to be solved may be formulated as the provision of an alternative RNA ligand to A-Beta(1-40) target protein.

In view of the fact that D1 (see abstract) already describes the enabling of designing specific RNA ligands against any target molecule, describes high-affinity RNA ligands against A-Beta1-40 fibrillar protein, and the screening for RNA ligands that bind to one form of the A-Beta1-40 amyloid protein (namely, beta-sheet conformation) and not to another form (namely, monomeric form); the design of alternative nucleic acid ligands binding to the monomeric form of the A-Beta1-40 protein is routine and cannot be considered to involve an inventive step, particularly in the absence of a surprising technical effect of said ligand.

Therefore, **claims 1 to 5** relating to the RNA ligand with SEQ ID NO: 1 against A-Beta1-40 monomeric target protein, and **claims 8 and 9** (relating to a vector comprising said RNA ligand and a host comprising said vector) are **not considered inventive.**

Claim 6 relating to alternative labelling of the RNA ligand is routine and therefore also **not** considered to involve an **inventive** step (see D1, p. 1584, left-hand-column, last paragraph).

The requirement of the additional feature of RNA ligand consisting of SEQ ID NO: 1 having constant flanking regions cannot be considered suitable for establishing the inventive step for the reasons that the use of flanking regions as point for attachment for PCR primers is well-known and frequently used in SELEX (see eg. D2, Table 1 which describes SELEX and T3 SELEX and T7 SELEX primers corresponding to SEQ ID NO: 56 and 106 of present application). Therefore, **claim 7** is **not** considered **inventive.**

Claims 10 to 18 relating to pharmaceutical compositions comprising said RNA ligand consisting of SEQ ID NO: 1, the use of said RNA ligand for manufacture of a medicament, the use of said RNA ligand for treating amyloid diseases (e.g. Alzheimer) and methods for treating or monitoring a patient suffering from a disease associated with amyloid formation by administering said nucleic acid ligand are not considered inventive because the prior art (see D1, p. 1584 first paragraph and p. 1587 right-hand-column or D3, cls 2, 10 and 12) already anticipates the therapeutic and diagnostic applications of RNA ligands for A-Beta1-40 proteins, particularly in the absence of a surprising technical effect of said ligand.

Therefore, **claims 1-18** (insofar they relate to invention 1) are **not inventive**.

V.2. INVENTION 61

The same reasoning applies to invention 61 which relates to a non-naturally occurring RNA ligand to native human Beta-2-microglobulin consisting of the nucleic acid sequence with SEQ ID NO: 61 (see items V.1.1 and V.1.2 above).

Owing to the way the inventions have been separated during search, unlike inventions 1 and 38, invention 61 embraces claims 28 and 29. Said claims pertain to the use of a binding motif comprising a peptide from human Beta-2-microglobulin that retains the ability to form amyloid fibrils as a target for selecting a nucleic acid ligand. This subject-matter is regarded as being novel over the cited prior art, however, it is the mere application of SELEX method to a protein known in the art.

It is the opinion of this Authority that the claims are no more than routine applications available to the skilled person at the time of filing. It is for this reason that said claims are not in conformance with Art. 33(3) PCT.

Therefore, **claims 1-4, 6-18 and 28 to 30** (insofar they relate to invention 61) are **novel** but are **not considered inventive.**

V.3. INVENTION 38

The prior art does not describe a non-naturally occurring RNA ligand to D-amino acid A-Beta1-40 protofibrillar protein consisting of the nucleic acid sequence with SEQ ID NO: 38, a vector comprising said RNA ligand, a host cell comprising said vector, a pharmaceutical composition comprising said RNA ligand, the use of said RNA ligand for manufacture of a medicament, and a method for the isolation of said RNA ligand.

Therefore, **claims 1-4, 6-18 and 31** (insofar they relate to invention 38) are considered **novel** (Art. 33(1) (2) PCT).

Nucleic acid ligand with SEQ ID NO: 38 is proven to be capable of binding to fibrillar proteins **and also** of blocking fibril formation. In view of the fact of the presence of an unexpected technical effect (namely, that said ligand inhibits fibril formation) and that said feature is not an inevitable consequence of the SELEX method, nucleic acid ligand with SEQ ID NO: 38 is considered to have an inventive character and thus **claims 1-4, 6-18 and 31** (insofar they relate to invention 38) are considered **inventive** (Art. 33 (3) PCT).

V.4. INVENTION 74

The same reasoning as for invention 38 applies to invention 74, which relates to a nucleic acid sequence molecule that binds to a Beta-2-microglobulin immature fibril protein target consisting of the nucleic acid sequence with SEQ ID NO: 74. Said nucleic acid ligand is able to block fibril formation.

Therefore, **claims 1-4, 6-18 and 28-30** (insofar they relate to invention 74) are considered **novel and inventive** (Art. 33(1) (2) (3) PCT).

INDUSTRIAL APPLICABILITY:

For the assessment of the present **claims 15 to 18** on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to a diagnostic method carried out on the living human or animal body.

FURTHER COMMENTS:

A. The **priority** appears to be allowable for all of the claimed subject-matter. The P-documents mentioned in the International Search Report therefore do not appear to be relevant.

B. Certain defects in the International Application:

* Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D2 and D3 are not mentioned in the description, nor are these documents identified therein.

* The relative term "substantially" used in claims 2 and 3 have no well-recognised meaning and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claims unclear, Article 6 PCT.

* The term "preferential binding affinity" is vague and imprecise, thereby resulting in lack of clarity of claim 5 (Article 6 PCT, see also PCT Guidelines, C-III, 4.3a).